PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2032195PC/OR	**FOR FURTHER ACTION** See Form PCT/IPEA/416	
International application No. PCT/FI2004/000776	International filing date *(day/month/year)* 17.12.2004	Priority date *(day/month/year)* 19.12.2003
International Patent Classification (IPC) or national classification and IPC See Supplemental Box		
Applicant Mobidiag Oy et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☒ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☒ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand 19-10-2005	Date of completion of this report 14-02-2006
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Terese Sandström/EÖ Telephone No. +46 8 782 25 00

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Cover sheet

INTERNATIONAL PATENT CLASSIFICATION (IPC):

C12Q 1/68 (2006.01)
C12N 15/11 (2006.01)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of:

☐ international search (Rules 12.3(a) and 23.1(b))

☐ publication of the international application (Rule 12.4(a))

☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-24 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 25-27 received by this Authority on 03-02-2006

pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-2 (figures 1-3) as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) -- see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority document has been received and examined.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. IV Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has, within the applicable time limit:

☐ restricted the claims

☐ paid additional fees

☐ paid additional fees under protest and, where applicable, the protest fee

☐ paid additional fees under protest but the applicable protest fee was not paid

☐ neither restricted the claims nor paid additional fees

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

☐ complied with

☒ not complied with for the following reasons:

The following separate inventions were identified:

1: Claims 1-11 and 16 directed to detection and identification of bacterial species involving the use of the universal primer pair SEQ. ID. NOs. 20 and 21 in combination with species-specific probes. Both the primers and probes hybridize to the rpoB gene.

2-12: Claims 12-15 directed to the species-specific probes SEQ. ID. NO. 1-19, which hybridize to a hyper-variable region that is situated near conserved regions in the rpoB gene. (One invention for each bacterial species that the probes may identify, i.e. one invention for the two probes capable of identifying H.influenzae, one invention for the two probes capable of identifying S.pyogenes etc.)

The present application has been considered to contain 12 inventions which are not linked such that they form a single general inventive concept, as required by Rule 13 PCT for the following reasons:

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4. Consequently, this report has been established in respect of the following parts of the international application:

☐ all parts

☒ the parts relating to claims Nos. 1-11 and 16

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box IV

The closest prior art has been identified as:
D1: WO0192573 A1
D2: US5786147 A
D3: WO0131061 A1
D4: WO9533851 A2
D5: WO03008645 A1
D6: WO03020972 A1
D7: WO03016534 A1

D1 discloses a method for identifying a Mycobacterium species using a combination of a universal primer pair and a species-specific probe, all hybridizing to the rpoB gene. (Page 5, line 21-25; page 5, line 29-page 6, line 7; page 7, line 16-page 8, line 4; table 2.)

Similar methods for combining universal primers with species-specific probes are disclosed in D2-D7. (D2: column 4, line 56-column 11, line 20; examples 1-2; D3: page 5, lines 1-18; page 7, line 15-page 10, line 8; page 13, line 6-page 21, line 23; D4: page 5, lines 3-13; page 15, lines 8-31;page 19, lines 6-17; table 2; claims; D5: page 5, line 22-page 9, line 15; page 11, line 15-page 20, line 8; D6: abstract; page 6, line 19-page 8, line 15; claims; D7:page 6, line 13-page 13, line 23; example 1.)

Invention 1:

From a comparison of the disclosure of D1-D7 and the technical features of claims the following technical features can be seen to make a contribution over this prior art: The use of the specific primer pair SEQ. ID. NO. 20 and 21 in combination with species-specific probes for identifying and detecting bacterial species.

This feature is hence considered as special technical feature in the sense of Rule 13.2 PCT.

The effect of this feature is an alternative method for detecting and identifying bacterial species.

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box IV

Inventions 2-12:

From a comparison of the disclosure of D1-D7 and the technical features of claims the following technical features can be seen to make a contribution over this prior art: Species-specific probes, disclosed in SEQ. ID. NOs. 1-19, for a number of different bacterial species.

This feature is hence considered as special technical feature in the sense of Rule 13.2 PCT.

The effect of this feature is species-specific probes for a number of different species. (One invention for each bacterial species that the probes may identify, i.e. one invention for the two probes capable of identifying H.influenzae, one invention for the two probes capable of identifying S.pyogenes etc.)

Even though the probes in inventions 2-12 might be used in the method in invention 1, they can not be considered "specially designed" for carrying out the method in invention 1, as other probes in the region are previously known. In addition, these probes can be used for any hybridization assay and the product claims do not imply a limitation as to a particular use.

The above analysis shows that the special technical features of invention 1 are neither the same as, nor corresponding to, those of inventions 2-12.

Consequently, neither the objective problem underlying the subjects of the claimed inventions, nor their solutions defined by the special technical features allow for a relationship to be established between the said inventions, which involves a single general inventive concept.

In conclusion, therefore, the claims are not linked by same or corresponding special technical features and define different inventions not linked by a single general inventive concept.

The application, hence does not meet the requirements of unity of invention as defined in Rule 13.1 and 13.2 PCT.

Since no fee was paid for a complete search, only invention 1 was searched during the Chapter I procedure. Hence only invention 1 is examined in this report.

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Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-11,16	YES
	Claims		NO
Inventive step (IS)	Claims	1-11,16	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-11,16	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Documents cited in the International Search Report:
D1: WO0192573 A1
D2: US5786147 A
D3: WO0131061 A1
D4: WO9533851 A2
D5: WO03008645 A1
D6: WO03020972 A1
D7: WO03016534 A1
D8: Khamis A. et al., "Usefulness of rpoB Gene Sequencing for Identification of Afipis and Bosea Species, Including a Strategy for Choosing Discriminative Partial Sequences", Applied and Environmental Microbiology, November 2003, pages 6740-6749
D9: Dahllöf I. et al., "rpoB-Based Microbial Community Analysis avoids Limitations Inherent in 16S rRNA Gene Intraspecies Heterogeneity", Applied and Environmental Microbiology, August 2000, pages 3376-3380
D10: Mollet C. et al., "rpoB sequence analysis as a novel basis for bacterial identification", Molecular Microbiology, 1997, Vol. 26, No. 5, pages 1005-1011

The present application relates to diagnosis of bacterial infections based on species-specific probes and broad-range primers. The species-specific probes originate from hyper-variable regions situated near the conserved sequences of the gene region encoding for RNA polymerase beta subunit, rpoB, of infection causing bacteria. The broad-range primers originate from conserved regions in the same gene. The problem to be solved by the present invention is to find a method for sensitive, effective, and species-specific identification of only the desired bacterial spices among various bacteria, which may be present in a clinical sample.

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

D1 discloses a method for identifying a Mycobacterium species using a combination of a universal primer pair and a species-specific probe. All hybridize to the rpoB gene. The universal primer pair gives rise to a PCR fragment of 157 base pairs. (Page 5, lines 21-25; page 5, line 29-page 6, line 7; page 7, line 16-page 8, line 4; table 2.)

D2 relates to detection and amplifying techniques utilizing oligonucleotide probes and primer and to the applications of these oligonucleotides for detecting enterobacteria. The methods are based on the use of nucleic acid sequences, which are strongly conserved for the enterobacterial species alone. These sequences are specifically defined in the rpoB gene, thereby enabling the enterobacterial family to be distinguished from other bacterial families. (Column 4, line 56-column 11, line 20; examples 1-2.)

Similar method for combining universal primers with species-specific probes are disclosed in D3-D7 (D3: page 5, lines 1-18; page 7, line 15-page 10, line 8; page 13, line 6-page 21, line 23; D4: page 5, lines 3-13; page 15, lines 8-31;page 19, lines 6-17; table 2; claims; D5: page 5, line 22-page 9, line 15; page 11, line 15-page 20, line 8; D6: abstract; page 6, line 19-page 8, line 15; claims; D7:page 6, line 13-page 13, line 23; example 1.) All these documents disclose methods for detection, identification and/or resistance analysis within single family only.

None of the cited documents disclose a method suitable for identification of a large number of bacterial pathogens simultaneously, i.e. the screening of a clinical specimen for a disease-causing bacteria. The primers disclosed in the cited prior art are derived from the species-specific regions of the rpoB gene or are derived from the antibiotic resistance genes and do not enable the detection of several bacterial species, which may be distantly related. Furthermore, none of the documents disclose a method, which is useful in bacterial diagnosis of infectious diseases, especially those causing respiratory tract infections and ear, nose and throat diseases.

The subject matter claimed in claims 1-11 and 16 differs from D1-D7 due to the specific broad-range primers disclosed in sequences SEQ. ID. NOs. 20 and 21. Hence, the subject matter claimed in claims 1-11 and 16 is novel.

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Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

These broad-range primer sequences have been based on a comparison of rpoB sequences from a large number of different bacteria, thereby having a broader detection range than the primers disclosed in D1-D7. With the broad-range primers of the present invention, the amplification of rpoB genes from phylogenetically different bacterial species is successful regardless of whether the bacterial species are gram-negative or gram-positive, even in the presence of large amount of human normal flora DNA. With these specific primers, it is possible to diagnose an infectious disease with unknown causative agent. In addition these primers give rise to a smaller PCR product, thereby increasing the effectiveness of the analysis.

Even though specific primers originating from conserved sequences of rpoB have been disclosed in the prior art, the development of a specific and sensitive diagnostic method for detecting and identifying bacterial species causing infection from a clinical sample cannot be considered as routine procedure for a skilled person, since unpredictability is highly characteristic in the field of biology and gene technology. Many factors affect the detection of a specific microorganism in a clinical sample, which contains DNA from many different sources. For instance, all biological samples of human origin contain DNA from normal flora. The effect of the normal flora on the analysis method cannot be predicted. Its effect can be unpredictable in cases, where the detection and identification of microorganisms, such as those causing infections, is base on conserved genes present in both eukaryotic and prokaryotic organisms. Additionally, clinical samples of human origin always contain human DNA, which similarly is a "contaminant" in cases where the detection and identification of microorganisms, such as those causing infections, is based on universally conserved genes.

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

In view of the above, the subject matter claimed in claims 1-11 and 16 is considered to involve an inventive step.

D8-D10 are considered to disclose the general state of the art.

To summaries, the subject matter claimed in claims 1-11 and 16 is novel and is considered to involve an inventive step. The subject matter claimed in claims 1-11 and 16 is considered to be industrially applicable.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO2004041841 A2	21.05.2004	04.11.2003	05.11.2002

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

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Claims (Amended on 3 February 2006)

1. A diagnostic method for detecting and identifying bacterial species causing infections from a clinical sample, characterized by

a) amplifying DNA isolated from said clinical sample using a mixture of DNA primers that comprises sequences which hybridize with the sequences that originate from conserved regions of *rpoB* genes encoding DNA directed RNA polymerase [EC:2.7.7.6] subunit B of bacterial species causing infections, said sequences comprising SEQ ID NOS: 20 and 21 and/or complementary sequences thereof and/or functional fragments thereof,

b) contacting the amplified DNA with a desired combination of oligonucleotide probe sequences that hybridize under normal hybridization conditions with hyper-variable regions situated near said conserved regions of *rpoB* genes encoding DNA directed RNA polymerase [EC:2.7.7.6] subunit B of bacterial species causing said infections, said sequences being bacterial species-specific under said hybridization conditions, and

c) detecting the formation of a possible hybridization complex.

2. The diagnostic method according to claim 1, characterized in that said infections causing bacterial species are bacterial species that cause human disease, particularly respiratory tract infections and/or ear, nose and throat diseases.

3. The diagnostic method according to claim 1 or 2, characterized in that said hyper-variable region is the hyper-variable region of the gene encoding the rpoB protein of a bacterial species selected from *Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Staphylococcus aureus, Legionella pneumophila, Corynebacterium diphteriae, Mycoplasma pneumoniae, Escherichia coli, Moraxella catarrhalis* and *Neisseria gonorrhoeae.*

4. The diagnostic method according to any one of claims 1 to 3, characterized in that the length of oligonucleotide probe sequences used in step b) is 15 – 30, more preferably 19 – 30, and most preferably 19 – 26 nucleic acids and are optionally labeled.

5. The diagnostic method according to any one of claims 1 to 4, characterized in that said combination of oligonucleotide probe sequences comprises all or a portion of SEQ ID NOS: 1 to 19, and/or complementary sequences thereof, or functional fragments thereof and preferably it comprises all of the SEQ ID NOS: 1 to 19.

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6. The diagnostic method according to claim 5, characterized in that said combination of oligonucleotide probe sequences is attached onto a solid support, preferably onto treated glass.

7. The diagnostic method according to claim 1, characterized in that the DNA isolated from the clinical sample in step a) is amplified using the polymerase chain reaction (PCR) and that the DNA amplified in step b) is contacted with bacterial species-specific oligonucleotide probes attached onto a solid support.

8. The diagnostic method according to claim 7, characterized in that suitably labeled nucleotides are used in the amplification of DNA isolated from a clinical sample in step a) to generate a detectable target strand and that the amplified and optionally labeled target DNA in step b) is contacted with a solid support, on which all bacterial species-specific oligonucleotide probes of SEQ ID NOS: 1 to 19 and/or complementary sequences thereof have been attached.

9. The diagnostic method according to claim 8, characterized in that the amplified and optionally labeled target DNA in step b) is contacted with a solid support, preferably treated glass, on which specific oligonucleotide probe sequences detecting one specified bacterial species or a few specified bacterial species causing infections have been attached, said sequences being selected from sequences shown in Table 3 and/or complementary sequences thereof.

10. The diagnostic method according to any one of claims 1 to 9, characterized in that the microarray technology is used in step c).

11. A DNA primer mixture, characterized by comprising sequences that hybridize with sequences of the conserved regions of *rpoB* genes encoding DNA directed RNA polymerase [EC:2.7.7.6] subunit B of bacterial species that cause infections, said mixture comprising SEQ ID NOS: 20 and 21 and/or complementary sequences thereof or functional fragments thereof.

12. An oligonucleotide sequence useful in the diagnosis of infection causing bacterial species, characterized in that it hybridizes under normal hybridization conditions with a sequence of a hyper-variable region that is bacterial species-specific and is situated near the conserved regions of *rpoB* genes encoding DNA directed RNA polymerase [EC:2.7.7.6] subunit B of bacterial species causing said infections, said oligonucloetide sequence being bacterial species-specific and said oligonucleotide sequence comprising one of the SEQ

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ID NOS: 1 to 19 or complementary sequences thereof functional fragments thereof.

13. The combination of oligonucleotide probe sequences useful in the diagnosis of infection causing bacterial species, characterized by comprising any combination of the SEQ ID NOS: 1 to 19 or complementary sequences thereof or functional fragments thereof.

14. The combination of oligonucleotide probes according to claim 13, characterized by comprising all of the SEQ ID NOS: 1 to 19.

15. The use of the combination of oligonucleotide probes according to claim 13 or 14 for the detection, identification, or classification of disease causing bacterial species.

16. A diagnostic kit for use in the diagnosis of infection-causing bacteria, especially those causing respiratory tract infections, characterized by comprising

a) a DNA primer mixture comprising sequences that hybridize with sequences of the conserved regions of *rpoB* genes encoding DNA directed RNA polymerase [EC:2.7.7.6] subunit B of bacterial species causing infections, especially bacterial species that cause respiratory tract infections, said mixture comprising SEQ ID NOS: 20 and 21 or complementary sequences thereof or functional fragments thereof,

b) a combination of bacterial species-specific oligonucleotide probe sequences, optionally attached on a solid support, comprising any combination of the SEQ ID NOS: 1 to 19 or complementary sequences thereof or functional fragments thereof,

c) positive and optionally negative control probe sequences, and optionally

d) reagents required in the amplification, hybridization, purification, washing, and/or detection steps.